Chiral Recognition in Gas-Phase Cyclodextrin: Amino Acid Complexes—Is the Three Point Interaction Still Valid in the Gas Phase?

Seonghee Ahn, Javier Ramirez, Gabriela Grigorean, and Carlito B. Lebrilla
Department of Chemistry, University of California, Davis, California, USA

The validity of the "three-point interaction" model is examined in the guest exchange reaction involving complexes of cyclodextrins and amino acids. The amino acid guest is exchanged in the gas phase in the presence of a gaseous alkyl amine. The net reaction is proton transfer between the protonated amino acid and the alkyl amine. The amino acid is lost as a neutral species. This reaction is sensitive to the chirality of the amino acid. Several amino acids are examined as well as the respective methyl esters to determine the role of the three interacting groups (ammonium, carboxylic acid, and side chain) in enantioselectivity. We find that the three-point interaction model is indeed valid in the gas phase. Enantioselectivity is optimal when two points of attraction and one repulsion is present in the gas-phase complex. The results are supported by molecular modeling calculations. A mechanism for the exchange is proposed. (J Am Soc Mass Spectrom 2001, 12, 278–287) © 2001 American Society for Mass Spectrometry

Chiral recognition is currently an active area of research in mass spectrometry. The interest in chiral recognition lies in its potential applications in areas as disparate as chemical synthesis, catalysis, enzyme mimetics, pharmaceutics, geochemistry, and biotechnology. For mass spectrometry, chiral discrimination is a challenging endeavor since enantiomeric isomers generally has identical mass to charge ratios (m/z), rendering them indistinguishable. Nonetheless, because of the importance of chiral compounds, chiral molecules continue to attract considerable attention [1–5]. The formation of noncovalently bound host–guest complexes in the gas phase have provided a useful method for discriminating enantiomers that takes advantage of the differences in the relative stability of the stereomeric complexes. Sawada and co-workers have illustrated various examples of stereomeric host–guest complexes formed by fast atom bombardment that have different stabilities [6–9]. A quantitative method was developed for evaluating the degree of chiral recognition by isotopically labeling one enantiomer and observing the relative intensity in a 1:1 mixture of enantiomers [10].

Ion–molecule reactions involving host–guest complexes have also been used as systems for chiral recognition. The enantioselectivity is obtained from the different rates for enantiomers in the gas-phase reactions. Several groups have observed enantiospecificity in protonated dimers and even trimers of tartrates. One of the earliest studies was reported by Fales on protonated dialkyltartrate dimers [11]. Other studies of the same or similar systems have followed, primarily by Nikolaev [12–15]. Enantioselectivity in the dimerization of host–guest complexes have been reported by Dearden [16, 17]. Enantioselectivity has been observed in this laboratory in the gas-phase deprotonation reactions of gas-phase cytochrome *c* ions using chiral amines [18, 19].

We recently reported a novel gas-phase guest exchange reaction involving complexes composed of cyclodextrins and protonated amino acids reacting with neutral alkyl amines [20]. The amino acid (AA) is displaced by the amine (B) in a guest exchange reaction to produce a new protonated complex $[CD:B + H]^+$ (Scheme **1**). More importantly, we observe enantioselectivity because the rate constants vary depending on the chirality (L or D) of the amino acids.

$$[CD:AA + H]^+ + B \rightarrow [CD:B + H]^+ + AA$$

Scheme 1

The reaction outlined in Scheme **1** is analogous to those found particularly in liquid chromatography. In chiral chromatographic separations, similar host–guest complexes are formed. The complexes are in equilibrium with solvent molecules that displace the analyte. The inclusion of the guest molecules inside the cyclo-

Address reprint requests to Carlito B. Lebrilla, Department of Chemistry, University of California, Davis, CA 95616. E-mail: cblebrilla@ucdavis.edu

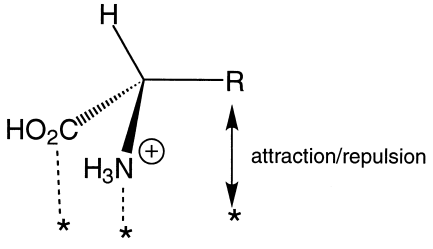
1044-0305/01/$20.00
PII S1044-0305(00)00220-8

Received May 10, 2000
Revised August 25, 2000
Accepted August 25, 2000

dextrin cavity is believed to provide the necessary requirements for chiral recognition and discrimination [21–24]. As we reported recently, inclusion is also a necessary requirement for chiral recognition in the gas phase [25].

Chiral discrimination requires that the host and guest form reasonably stable complexes. It is the cooperative interaction of several weak forces such as dipole–dipole, hydrophobic, electrostatic, van der Waals, and hydrogen bonding that lead to molecular recognition and differentiation. The "three-point attachment" model is the combination of these interactions and has been long used in the condensed phase to understand enantioselectivity [26, 27]. In later years, this model has been refined to be called the "three-point interaction" model to describe more generally the nature of the interactions [28]. Although controversies arose on whether it is still valid particularly in light of more complex enzymatic systems, the model has remained essentially intact [29, 30].

In this report, we illustrate the validity of the three-point interaction model to describe enantioselectivity in gas-phase host–guest complexes. Scheme **2** illustrates these interactions for amino acids. The α-amino acids offer ideal systems for study. Two attractive interactions are present involving the protonated amine and the carboxylic acid group. The interaction of the side chain can either be attractive or repulsive depending on the functional group. The magnitude of the interaction can also be varied with the size and the type functional groups. These interactions and the role of the hosts will be explored in this report.



Scheme 2

Experimental

Materials

All D-amino acids, heptakis-(2,3,6)-tri-O-methyl-β-cyclodextrin (β-CD), maltoheptaose, *n*-propylamine, 2-butylamine, pyridine and (R)- and (S)-1-amino-2-propanol were obtained from Sigma Chemical (St. Louis, MO) and used without further purification. L-amino acids were obtained from Research Plus (Denville, NJ). The permethylated maltoheptaose, maltohexaose, and maltopentaose were synthesized in this laboratory as described below. The silica tubing used to manufacture the microspray tips was purchased from Polymicro Technologies (Phoenix, AZ).

Methylation of Linear Oligosaccharides

The methylation of the linear oligosaccharides was carried out following the method of Ciucanu et al. [31]. About 20–25 mg of the oligosaccharide was dissolved in 2.0 mL of DMSO along with 200 mg of NaOH. To this mixture, 1.0 mL of CH_3I was added slowly and the solution was gently stirred at room temperature for 2 h. After the reaction, the insoluble inorganic material was removed by filtration. Subsequently, 5.0 mL of chloroform was added and the inorganic salts were extracted three times sequentially with water, aqueous sodium thiosulfate solution, and water. The chloroform was removed by evaporation under vacuum.

Esterification of Amino Acids

The esterification of amino acids was carried out as described by Hoogwater and Peereboom [32]. Briefly, the procedure involved the addition of 0.1 mL of thionyl chloride to 1.0 mL of methanol at -10 °C. A 0.11 mol amount of amino acid was added and the mixture was refluxed for a period of 2 h. The reaction mixture was then evaporated under vacuum and the residue recrystallized in a methanol–diethylether solution.

Guest-Exchange Reactions

All mass spectrometry experiments were performed on a home-built external source electrospray ionization Fourier transform mass spectrometry equipped with a 5.1 tesla superconducting magnet (Oxford Instruments, Witney, England). The details of the instrument have been published elsewhere [33, 34]. The solutions were electrosprayed by applying a voltage of 1.5–2.5 kV at the liquid junction on the base of the microspray tip. The microspray tips were manufactured from silica tubing with an o.d. of 150 μm and an i.d. of 25 μm. The typical flow rates ranged from 10 to 15 μL/h.

All amino acid and oligosaccharide stock solutions (0.01 M) were prepared in a 50/50 (V/V) water/methanol solution. The cyclodextrin:amino acid complexes were prepared by mixing the cyclodextrin with a 10- to 50-fold excess of the desired amino acid. The excess amino acid increased the signal intensity of the complex. The final concentration of cyclodextrin in the solution was 1.0×10^{-5} M. The isolation steps were carried out using a series of rf bursts and sweeps at frequencies corresponding to the unwanted masses.

The isolated complex was then allowed to undergo a guest exchange reaction with an amine that was previously leaked into the analyzer cell. Gaseous amines were first purified on the vacuum manifold by several freeze–thaw cycles and then leaked into the analyzer cell with pressures between 1 and 6×10^{-7} torr as determined by an uncalibrated ion gauge. The appearance of the exchange product was monitored as a function of time. Rate constants (k) were obtained from the slopes of the pseudo-first-order rate plots ($\ln I/I_0$ vs.

t, where I is the intensity of the complex at time t and I_0 is the sum of the intensities of the product and starting complex). The largest source of error in determining rate constants is in the accurate determination of the pressure. Our current data system has limited access to the very low mass range prohibiting us from performing the standard pressure calibration reactions involving methane. We have performed pressure calibration with published deprotonation reactions of proteins, however the consistency was not satisfactory. We therefore caution the reader that the absolute rates will not be accurate. However, the important number in this study is the selectivity, which we define as the ratio k_L/k_D. In this ratio, any deviation in pressure from the "true" value is completely eliminated. The deviation in selectivity values is less than 10% as determined from multiple determinations.

Molecular Modeling

The cyclodextrin, linear oligosaccharides, and amino acids structures were constructed and optimized using the Insight II builder module. The protonated oligosaccharide:amino acid complexes were formed by merging the respective sugars and amino acids. Calculations of the complexes were started with fully optimized oligosaccharide host and amino acid structures. During the simulation, the structures of both the amino acids and the hosts were allowed to fully optimize. In the first set of calculations, the amino acid was placed near the upper, wider rim of the CD molecule (nonincluded complex). The complex was heated to 600 K for 400 ps. At every 8 ps, a structure from the trajectory was captured and annealed in steps of 100 to 0 K. The heating/annealing cycles helps avoid local minima and provides the best solution to the global minimum. This resulted in 50 annealing simulations with a corresponding number of structures. Generally, several structures with very similar energies were obtained. Only the lowest energy structure of each enantiomer is presented. However, all the structures within 5 kcal/mol of the lowest energy structure were found to share the same structural features. In the second set of calculations, the amino acids were placed inside the CD cavity in the starting geometry (included complex).

Molecular modeling simulations with methylated maltoheptaose, the linear analog of CD, and maltopentaose were carried out in a similar fashion. The host molecule was again fully optimized. During optimization, the isolated linear oligosaccharide adopted a helical structure that produced a "cavitylike" environment (structure not shown). For direct comparisons, the oligosaccharide was oriented with its secondary hydroxyl (or rather methoxyl) groups pointing upwards to resemble the wider rim of cyclodextrin. The amino acid was placed close to the upper rim to resemble the nonincluded complex. For the "included" complexes, the amino acid were placed inside the "quasicavity" of the coiled linear host.

Table 1. Rate constants[a] and selectivity, defined by the ratio k_L/k_D, for amino acids complexed to β-cyclodextrin. Due to the extreme slow reaction rates with pyridine, it was difficult to obtain rate constants and selectivities

Amino acids		*n*-Propyl amine (889.0)[b]	2-Butyl amine (895.7)[b]	Pyridine (898.1)[b]
Ala	k_L	2.4	1.6	<0.001
	k_D	1.5	1.3	<0.001
	k_L/k_D	**1.6**	**1.2**	**N/A[c]**
Val	k_L	3.4	1.5	<0.001
	k_D	1.1	1.1	<0.001
	k_L/k_D	**3.1**	**1.4**	**N/A[c]**
Ile	k_L	1.0	0.31	<0.001
	k_D	0.26	0.19	<0.001
	k_L/k_D	**3.8**	**1.6**	**N/A**
Leu	k_L	0.50	—	<0.001
	k_D	0.14	—	<0.001
	k_L/k_D	**3.6**	**—**	**N/A[c]**
Phe	k_L	1.4	0.16	<0.001
	k_D	1.7	0.17	<0.001
	k_L/k_D	**0.82 (1.2)**	**0.94 (1.1)**	**N/A[c]**
Tyr	k_L	0.019	—	<0.001
	k_D	0.029	—	<0.001
	k_L/k_D	**0.66 (1.5)**	**—**	**N/A[c]**
Ser	k_L	0.064		
	k_D	0.052		
	k_L/k_D	**1.2**		
Thr	k_L	0.12		
	k_D	0.19		
	k_L/k_D	**0.63 (1.6)**		
Asp	k_L	0.02		
	k_D	0.01		
	k_L/k_D	**2.2**		
Glu	k_L	0.01		
	k_D	0.005		
	k_L/k_D	**1.9**		

[a]All rate values $\times 10^{-11}$ cm^3/molecule s. Values in parentheses are the inverse of k_L/k_D.
[b]GB values are obtained from Hunter et al. [35].
[c]N/A: Experiments were performed by selectivity values and could not be obtained.

Results

The Reactions of Amino Acids Complexed to β-Cyclodextrin Host

The amino acids in Table 1 can be divided into several groups depending on the nature of their side chains. The "alkyl" amino acids constitute the group including Ala, Val, Ile, and Leu. The "aromatic" amino acids include Phe and Tyr. The "hydroxyl" include Ser and Thr, whereas the "acidic" include Asp and Glu. The "basic" amino acids which include Lys, His, and Arg were not examined because the resulting complexes were too unreactive with the chosen alkyl amines. Diamines are currently being used to study these compounds.

Within the alkyl amino acids, enantioselectivity (k_L/k_D) with *n*-propylamine increases with the size of the side chain. The enantioselectivity increases rapidly from 1.6 for Ala to 3.1 for Val and 3.6 for Leu. These results were provided and discussed previously as is

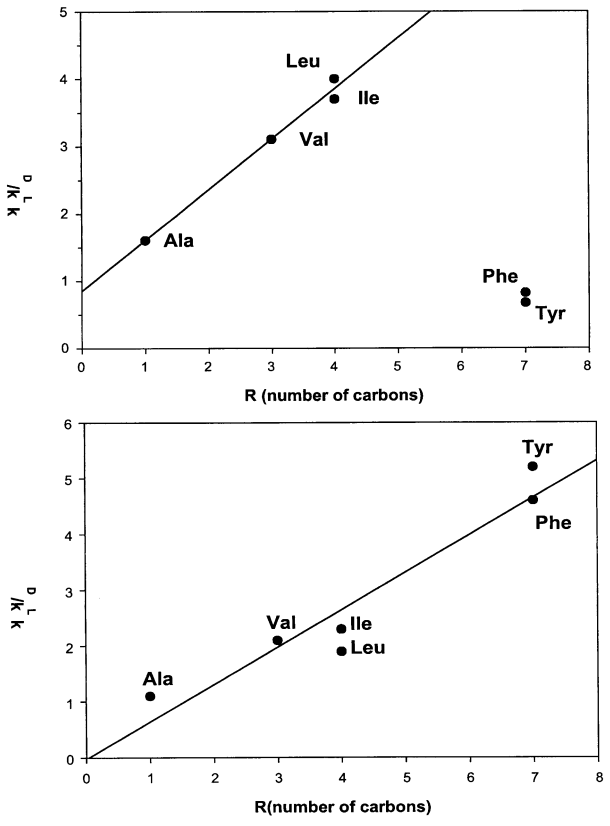


Figure 1. Plot of selectivity (k_L/k_D) as a function of the number of carbons in the side chain (R) of the amino acid for the hosts (**a**) permethylated-β-cyclodextrin and (**b**) permethylated-maltoheptaose. Enantioselectivity tends to increase with increasing number of carbons on the side chain of the alkyl amino acids. Phe and Tyr do not follow this trend with permethylated-β-CD but do so with permethylatedmaltoheptaose.

the graphical depiction in Figure 1a [25]. They are shown only for comparison.

The general trend of the amino acids with alkyl side chains is easily rationalized by the three-point interaction. As the alkyl group gets bigger, the repulsive interaction also increases. Because the attractive interactions of the amino and carboxylic termini have to be reconciled with the increasing steric repulsion, the enantiomers are forced to adopt specific conformations that lead to enantioselectivity [25]. We have proposed, based on experimental and theoretical evidence, that all gas-phase cyclodextrin:amino acid complexes involve inclusion structures [25]. For the alkyl amino acids, molecular modeling simulations predict that the orientation of the alkyl side chain relative to the central cavity of cyclodextrin differ significantly for each isomer. Differences in binding between the two enantiomers and the size of the alkyl group cooperate to increase selectivity by hindering the incoming *n*-propyl amine from approaching the protonated amino acid. We believe that access to the central cavity by the incoming alkyl amine is an important factor in the selectivity.

The aromatic amino acids, Phe and Tyr, do not seem to follow the same trend as the alkyl amino acids. The selectivity is diminished and even reversed (k_L/k_D = 0.82 and 0.66, respectively). Although the aromatic side chain differs somewhat electronically from the alkyl amino acids, their effect on selectivity should still be based on steric interactions rather than electronic ones. Figure 1a shows the plot of the selectivity as a function of number of carbons on the side chain R with the β-CD host. Based solely on the number of carbons, Phe and Tyr should have selectivities greater than 4.0. One can argue that the phenyl group is somewhat smaller than the alkyl side chains of Leu and Ile. However, that would decrease the selectivity to some degree but not to the amount observed.

The behavior of the aromatics appear unique compared to the alkyl amino acids. However, the behavior of Phe and Tyr is consistent with the alkyl amino acids if all of the amino acid complexes are inclusion complexes. The finite size of the cyclodextrin cavity suggests that a specific amino acid size could complement the cyclodextrin cavity size to yield the optimal selectivity. Once this size is reached, further increase in size will either produce no further increase in selectivity or may even decrease it. For β-cyclodextrin, this point is reached with Phe and Tyr. Molecular modeling simulations indicate that D- and L-Phe tend to form inclusion complexes that are sterically compromised due to the size of the phenyl ring (Figure 2). Steric interactions force the enantiomers of Phe to adopt similar conformations inside the cyclodextrin cavity, thereby reducing enantioselectivity. Similar results from molecular modelling (MM) calculations were obtained with Tyr [25].

Phe and Tyr are both significantly less reactive than the alkyl amine acids, albeit Phe complexes react nearly two orders of magnitude faster than the corresponding Tyr. There are two major reasons for low reactivity of Tyr. First, the gas-phase basicity of Tyr (892.1 kJ/mol) [35] is greater than that of Phe (888.9 kJ/mol) [35] making the overall proton transfer reaction unfavorable. Second, the hydroxyl group of tyrosine interacts with the upper rim of cyclodextrin as predicted by MM calculations (Figure 2) [25]. These hydrogen bonding interactions hinder the incoming amine from entering the cavity via the upper rim thereby decreasing reactivity significantly.

The hydroxyl groups on the side chain of Ser and Thr are capable of forming attractive hydrogen bonding interactions with the rim and interior of cyclodextrin making all three interactions attractive. Both Ser and Thr exhibit reaction rates slower than the similarly sized alkyl amino acids. The rate constants for Ser and Thr are over an order of magnitude less than Val. Although hydrogen bonding interactions decrease the rate of reaction, they do not necessarily increase selectivity. The size of the R groups in Ser and Thr fall roughly between those of Ala and Val. Based only on the size of the side chains, one would expect chiral

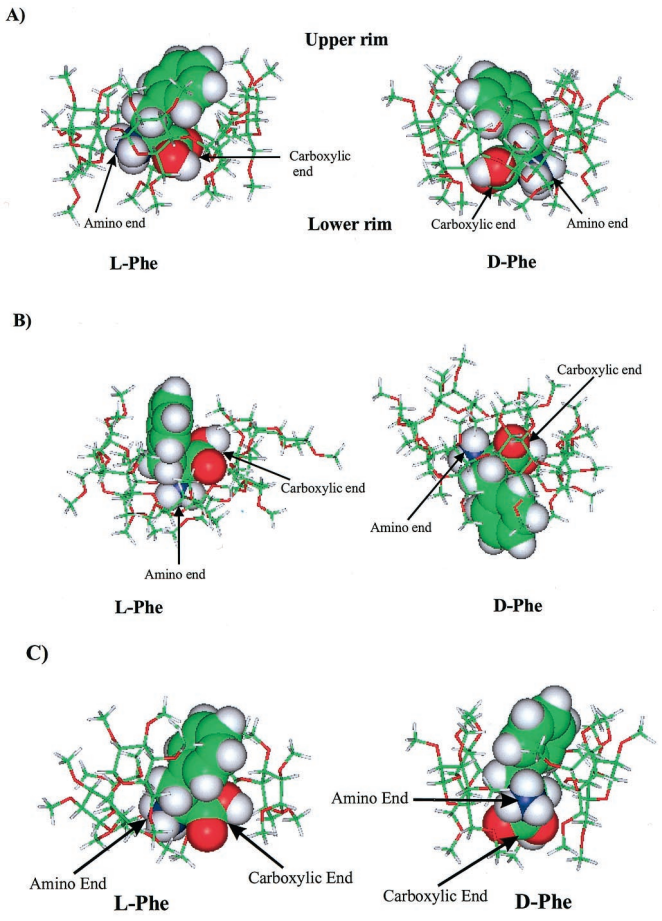


Figure 2. The lowest energy structures for enantiomeric pairs of Phe with (**a**) b-CD ([CD:Phe + H]$^+$), (**b**) maltoheptaose ([Hep:Phe + H]$^+$), and (**c**) maltopentaose ([Pen:Phe + H]$^+$). Structures were obtained using an Insight II/Biosym program package. Maltoheptaose and maltopentaose are oriented similarly to CD with the 2′ and 3′ positions pointing upwards to resemble the wider rim of CD. The enantiomeric pairs of Phe adopt similar orientation within the CD molecule but produce very distinct interactions with maltoheptaose.

selectivities between 1.6 and 3.1, respectively. However, the observed selectivities are only 1.2 for Ser and 0.63 (inverse 1.6) for Thr. Apparently, the presence of additional attractive interactions tends to diminish selectivity rather than enhance it. At this time, we have no explanation for why the selectivity of Thr is reversed. The molecular modeling yield only differences in binding and the exact mechanism for this reaction is still unknown.

Acidic amino acids are capable of even stronger hydrogen bonding interactions through their side chains. Indeed, the reactivity of Asp and Glu is even lower, as much as two orders of magnitude, than that of alkyl amino acids of comparable size. For example, the rate constant for L-Asp is significantly smaller than L-Val, despite its only slightly smaller size. Similarly, the chiral selectivities of these amino acids are lower than the alkyl amino acids of similar size. The results are in agreement with those for Ser and Thr and suggest that increasing the number of attractive interaction from two to three by the formation of an additional hydrogen bonds decreases both the rate of the reaction and the enantioselectivity.

Reactions with other achiral amines. The rate constants for guest exchange reactions involving a more basic and at the same time more hindered alkyl amine, 2-butylamine, are similar in magnitude to *n*-propylamine. Enantioselectivity is decreased with 2-butylamine in the few representative amino acids examined (Table 1). The amino acid with alkyl and phenyl side chains all decrease in selectivity with 2-butylamine. The selectivity for Ala decrease by about 25% from 1.6 to 1.2. For Val and Ile, they decrease by more than 50% (3.1 to 1.4 for Val and 3.7 to 1.6 for Ile).

The reactions with pyridine were extremely slow, making it very difficult to obtain reliable rate constants. The reaction rate constants are at least three orders of magnitude slower than that of both *n*-propylamine and 2-butylamine. This is not surprising as pyridine is the same size as benzene. Its size makes it difficult to enter the cavity when the cavity is already occupied. This

Table 2. Rate constants[a] and selectivity (k_L/k_D) of selected amino acids with (R)- and (S)-1-amino-2-propanol

Amino acids		(R)-1-A-2-P (217.0)[b]	(S)-1-A-2-P (217.0)[b]
Ala	k_L	10.8	12.3
	k_D	6.2	8.1
	$\boldsymbol{k_L/k_D}$	**1.7**	**1.5**
Val	k_L	8.4	8.9
	k_D	4.1	4.8
	$\boldsymbol{k_L/k_D}$	**2.0**	**1.9**
Ile	k_L	6.9	7.4
	k_D	3.5	3.0
	$\boldsymbol{k_L/k_D}$	**2.0**	**2.5**
Leu	k_L	4.7	8.5
	k_D	1.6	2.5
	$\boldsymbol{k_L/k_D}$	**3.0**	**3.4**
Phe	k_L	2.1	2.3
	k_D	2.2	2.2
	$\boldsymbol{k_L/k_D}$	**1.0**	**1.0**
His	k_L	<0.001	0.014
	k_D	<0.001	<0.001
	$\boldsymbol{k_L/k_D}$	**N/A**[c]	**N/A**[c]

[a]All rate values $\times 10^{-11}$ cm^3/molecule s. Values in parentheses are the inverse of k_L/k_D.
[b]GB values are obtained from Hunter et al. [35].
[c]N/A: Experiments were performed by selectivity values and could not be obtained.

notion is consistent with the known cavity size of β-cyclodextrin, which is about the same dimension as a benzene molecule.

Reactions with chiral amines. It was believed that a chiral alkyl amine could further enhance selectivity. In a previous publication, we reported the gas-phase deprotonation reaction of cytochrome *c* that was sensitive to the chirality of 1-amino-2-propanol, the reagent base [18, 19]. We investigated these two compounds further to observe the effect of chirality in the reagent alkyl amine. 1-Amino-2-propanol is more basic than *n*-propyl amine but it has approximately the same steric bulk. It was also believed that the alcohol group could provide an orienting effect by interacting with the rim and the internal cavity of cyclodextrin.

1-Amino-2-propanol is slightly more reactive than *n*-propylamine. Enantioselectivity is apparently not enhanced by the chiral alkyl amines (Table 2). All selectivity values are lower for the chiral alkyl amine than for *n*-propylamine, but the general trend in enantioselectivity with the chiral amines is similar to that obtained with *n*-propylamine. Selectivity increases from Ala (1.7) to Val (2.0) and Leu (3.0) but decreases significantly for Phe (1.0). All selectivity values are generally smaller for 1-amino-2-propanol. For example, the enantioselectivity (k_L/k_D) of Val is 2.0 with (R)- and 1.9 with (S)-1-amino-2-propanol, compared to 3.1 with *n*-propylamine. Similarly, the selectivity for Leu is 3.0 and 3.4, respectively, for the R and the S-1-amino-2-propanol compared to 3.6 for *n*-propylamine. We also find no significant differences between the reactivities of R- and S-1-amino-2-propanol. The largest difference in selectivities is found for Ile where the selectivity for the S is slightly more than the R (2.0 versus 2.5).

Enantioselectivity With Linear Oligosaccharide Hosts

In our effort to find a better host for enantioselectivity, we studied the linear analogs of cyclodextrin, namely the permethylated maltoheptaose, maltohexaose, and maltopentaose. This study allowed us to investigate the attributes of linear oligosaccharides as potential chiral selectors. It was not known whether linear oligosaccharides can act as hosts in gas-phase guest-exchange reactions. Even if the complexes were formed, it was not clear whether they would exhibit enantioselectivity. However, as hosts the linear oligosaccharides offered greater experimental and structural flexibility. The size (length) of the linear hosts can be varied to a larger degree compared to the limited number of differently sized cyclic oligosaccharides.

Indeed, linear oligosaccharides produce protonated complexes that undergo guest exchange reactions. The rate constants are of the same magnitude as the cyclic hosts. Enantioselectivity is observed and summarized in Table 3. The cyclodextrin results are also included in the same table for easier comparisons. Enantioselectivity is significantly diminished when maltoheptaose is used as the host for the alkyl amino acids. Enantioselectivity decreased by more than 30% for Ala, Val, and Ile. Leu decreased by more than 50%. Conversely, enantioselectivity increased significantly for Phe (from 0.82 to 4.6) and Tyr (from 0.66 to 5.2). A plot of enantioselectivity (k_L/k_D) versus R (the number of carbons in the side chain) now shows a linear relationship (Figure 1b), where selectivity increases with the number of carbon atoms on the side chain.

A further decrease in enantioselectivity was observed for all the amino acids in Table 3 with permethylated maltopentoase (an oligomer with five glucose units). Val, for example, decreases from 2.1 for maltoheptaose to 1.0 for maltopentaose. Ile similarly decreases from 2.3 to 1.5. For completeness, maltohexaose (with six glucose units) was examined with an alkyl and an aromatic amino acid. The selectivity of Leu decreases from maltoheptaose (1.9) to maltohexaose (1.4) and maltopentaose (1.8). Phe decreases from 4.6 to 1.2 for both maltohexaose and maltopentaose.

In order to gain insight into the molecular interactions between the host and the amino acids, we performed molecular dynamics simulations with amino acids and the maltoheptaose and maltopentaose hosts. The results for Phe are shown in Figure 2. In Figure 2, all three hosts are oriented similarly to facilitate comparison. Carbons 2 and 3 of the host are oriented as the upper rim and carbon 6 is oriented as the lower rim. The oligosaccharide host is represented in frame mode, whereas the amino acids are in space filling mode.

Table 3. Rate constants[a] and selectivity (k_L/k_D) of selected amino acids with CD and linear oligosaccharide hosts. All hosts are permethylated

Amino acids		Met-β-CD	Met-Heptaose	Met-Hexaose	Met-Pentaose
Ala	k_L	2.4	3.4	—	4.2
	k_D	1.5	3.1	—	3.9
	k_L/k_D	**1.6**	**1.1**	**—**	**1.1**
Val	k_L	3.4	3.4	—	3.2
	k_D	1.1	1.6	—	3.1
	k_L/k_D	**3.1**	**2.1**	**—**	**1.0**
Ile	k_L	1.0	3.0	—	1.6
	k_D	0.26	1.3	—	1.1
	k_L/k_D	**3.8**	**2.3**	**—**	**1.5**
Leu	k_L	0.50	2.5	2.9	1.5
	k_D	0.14	1.3	2.1	0.85
	k_L/k_D	**3.6**	**1.9**	**1.4**	**1.8**
Phe	k_L	1.4	0.63	1.1	0.86
	k_D	1.7	0.14	0.89	0.69
	k_L/k_D	**0.82 (1.2)**	**4.6**	**1.2**	**1.2**
Tyr	k_L	0.019	0.026	—	—
	k_D	0.029	0.005	—	—
	k_L/k_D	**0.66 (1.5)**	**4.9**	**—**	**—**

[a]All rate values $\times 10^{-11}$ cm^3/molecule s. Values in parentheses are the inverse of k_L/k_D.

The maltoheptaose host still interacts intimately with its amino acid guests (Figure 2b). The molecule wraps around the guest forming a quasi-inclusion complex that appears in many respect similar to the cyclodextrin complexes. In the included CD complex of Phe, the amino acid is forced to maintain the same interaction for both enantiomers. This is readily observed from the orientation of the phenyl group, which sticks out of the upper rim for both enantiomer of Phe when CD is the host. However, for the maltoheptaose complex of Phe, each enantiomer has clearly distinct interactions. In the L-Phe, the phenyl group is penetrating the "upper rim," whereas with D-Phe the phenyl group is penetrating the "lower rim."

The shorter chain lengths of maltohexaose and maltopentaose are less effective at complexing the amino acids. The lowest energy structures for D- and L-Phe with maltopentaose are shown in Figure 2c. The shorter maltopentaose is not able to fully include the Phe guest as the longer maltoheptaose. This affects the viability of the three-point interaction by not allowing one of the interactions to manifest fully.

Enantioselectivity of Modified Amino Acids

Amino acids were esterified, as described in the Experimental section, to effectively replace the attractive hydrogen bonding interaction between the carboxylic acid and the cyclodextrin rim with repulsive steric interactions. This limits the host–guest interaction to only a single attractive interaction for the alkyl amino acids and Phe. The summary of the selectivity values for the β-CD and the maltoheptaose complexes is provided in Table 4. The esterified amino acids react faster than their native counterparts regardless of the host. For example, the methyl esters of L- and D-Ala complexed to β-CD have rate constants 2.0 and 1.6 times greater than the native. The difference for Leu is even greater with the ester being 4.2 and 6.9 times greater than the native. The same increase in reactivity is observed for the maltoheptaose host.

A comparison of the selectivity values in Table 4 yields a few trends in the enantioselectivity. For cyclodextrin, enantioselectivity is decreased in the ester of amino acids with large alkyl side chain such as Val (3.1 to 0.9) and Leu (3.6 to 0.76). For Phe, there is a slight increase in selectivity with an inversion of reactivity (0.82 to 1.5). At this time, it is difficult to interpret the meaning of these selectivity inversions. Molecular modeling predicts only differences in interactions between enantiomeric pairs. How these differences translate to differences in rates has yet to be determined. The smallest chiral amino acid, Ala, increases in selectivity when it is converted to the ester (1.6 to 2.4). The largest amino acid, Tyr, increases in selectivity between the native and the ester; its k_D/k_L ratio increases from 1.5 to 3.0.

Enantioselectivity is generally diminished when malthoheptaose is used as the host. The selectivity of alanine stays nearly the same relative to the native, from 1.1 to 1.3 for the ester. All other amino acids decrease including Val (2.1 to 0.93), Leu (1.9 to 1.1), Phe (4.6 to 0.66), and Tyr (5.2 to 1.6). The linear oligosaccharide, maltoheptaose, provides a better host for isolating the effects of esterification because the effect of cavity size is minimized. With this host, reducing the number of attractive interaction from two to one increases the rates of reaction and clearly decreases enantioselectivity. The results obtained with cyclodextrin are complicated by a "cavity-size effect." For example, the selec-

Table 4. Rate constants[a] and selectivity (k_L/k_D) of selected amino acid esters with permethylated β-CD and maltoheptaose as hosts

Amino acid esters		Met-β-CD	Met-Heptaose
Ala	k_L	4.8	5.7
	k_D	2.0	4.5
	k_L/k_D	**2.4**	**1.3**
Val	k_L	1.9	3.9
	k_D	2.1	4.2
	k_L/k_D	**0.90 (1.1)**	**0.93 (1.1)**
Leu	k_L	2.2	3.4
	k_D	2.9	3.2
	k_L/k_D	**0.76 (1.3)**	**1.1**
Phe	k_L	2.8	2.7
	k_D	1.9	4.1
	k_L/k_D	**1.5**	**0.66 (1.5)**
Tyr	k_L	0.79	0.39
	k_D	2.4	0.25
	k_L/k_D	**0.33 (3.0)**	**1.6**

[a]All rate values $\times 10^{-11}$ cm^3/molecule s. Values in parentheses are the inverse of k_L/k_D.

tivity of alanine is increased for the ester because the size of the molecule is increased giving it a more complementary size for inclusion and chiral differentiation.

Discussion

The Role of the Three-Point Interaction in the Guest Exchange Reaction

The three-point interaction model, which is generally used to understand enantioselectivity in the condensed phase, is also operative in the gas phase. For optimizing enantioselectivity, there is a preference for two attractive and one repulsive over either one attractive or three attractive interactions. For example, Asp and Glu with three attractive hydrogen bonding interactions exhibit only moderate selectivity compared to similarly sized amino acids such Ala and Val with two attractive interactions. Indeed the size of the alkyl side chain is a better predictor of enantioselectivity for the alkyl amino acids. Similarly, producing the ester and reducing the attractive interaction to only one decreases enantioselectivity. The conversion of the amino acids to the corresponding methyl esters generally decreases enantioselectivity. This behavior is strongly evident in the reaction of amino acid esters complexed to permethylated maltoheptaose.

The importance of the two-point attraction with a third being repulsive has long been known. The "rocking tetrahedron" proposed by Sokolov and Zefirov to describe chiral discrimination in enzymatic reaction points to the importance of two attractive interactions [28]. In the condensed phase, the third interaction can be productive whether it is repulsive or attractive [21]. However, in gas-phase systems it is clear that the third interaction is preferably repulsive.

The host plays more than a passive role in enhancing the host–guest interaction. The differences in reactivity between the linear and cyclic analogs provide further evidence for the presence of gas-phase inclusion complexes. Although cyclodextrin is a relatively flexible molecule, it is rigid compared to the linear oligosaccharides. Some rigidity is important for enhancing enantioselectivity. Lack of rigidity in the macromolecular host allows the guest to find multiple favorable interactions that reduce the steric hindrance at the most enantioselective sites diminishing enantioselectivity. Still et al. pointed out that the limited conformational flexibility of the macrocycles they studied was a key element in obtaining enantioselectivity [36]. Rigidity of the host is an essential requirement in the "three-point interaction model" as it ensures that the diastereomeric complexes are conformationally and thermodynamically different [30, 37]. Thus, a more rigid host usually exhibits higher selectivity than a less rigid one.

The magnitude of the selectivity is improved by a "cooperativity effect" between the host and the guest. This is particularly true for cyclodextrin where the fixed ring size imposes strong conformational constraints. Steric repulsion is exacerbated by the formation of inclusion complexes, especially with amino acids having bulky side chains. To reconcile all the interactions between the host and the guest, the enantiomers adopt different conformations inside the cyclodextrin cavity. The cooperativity effect works well for Ile and Leu with cyclodextrin hosts. These compounds exhibit the largest selectivity. When the guest is too large, the cooperativity effect is again diminished. Phe and Tyr with bulky side chains are forced by the finite cavity size of the CD host to adopt similar conformations thereby decreasing selectivity.

In contrast to the rigidity of the cyclodextrin host, linear oligosaccharides provide an adjustable cavity that readily accommodates large guests. Maltoheptaose envelopes the amino acid producing a quasi-inclusion complex that is analogous to that present in cyclodextrin. The minimum size of this cavity is larger than the corresponding β-CD, and it is expandable to accommodate even larger sizes. Thus smaller amino acids exhibit less selectivity, whereas large amino acids such as Phe and Tyr produce greater selectivity.

Decreasing the size of the linear host decreases the selectivity as would be expected based on the three-point interaction model. The molecular modeling of protonated Phe complexed to permethylated maltopentaose predicts the host to encircle only about 70% of the molecule. The partial inclusion attenuates the intensity of the three-point interaction decreasing the selectivity for all amino acids.

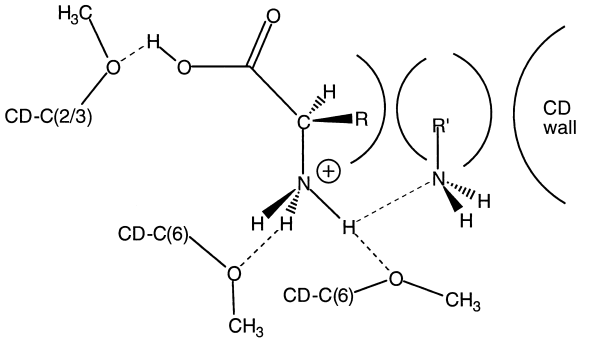
Mechanism of the Guest Exchange Reaction

Because the amino acid is included in the cyclodextrin, the alkyl amine must enter the cyclodextrin

cavity through either the upper or lower rim. The ability of the alkyl amine to penetrate the rim accounts for the magnitude of the exchange rate. It explains why pyridine, a "dialkyl amine," is essentially unreactive. The sterically hindered amine finds it difficult to enter the already occupied cavity. From the molecular dynamics calculation, we find that the ammonium group for each amino acid is coordinated to the lower rim. The C(6)–OCH_3 groups of the lower rim are more flexible and able to coordinate better with the ammonium group. This coordination distorts the lower rim by attracting the methoxyl oxygen toward the ammonium group, effectively blocking the approach through the lower rim. This means that the approach of the alkyl amine through the upper rim is likely to be preferable.

Attack through the upper, wider rim is consistent with the experimental results. Enantioselectivity would not be observed if the alkyl amine attacks through the lower rim and directly deprotonates the ammonium group. We would similarly not observe selectivity to increase with the increasing size of the alkyl side chain of the amino acid. Instead, because the R group is oriented towards the center of the cavity, it slows the reaction and increases selectivity when it gets bigger. The slow reaction of Tyr is also consistent with the attack through the wider rim [25]. MM simulations predict that the hydroxyl group of Tyr interacts with the upper rim. This interaction fixes the phenyl group and blocks the approach thereby decreasing the rate of reaction.

In essence guest exchange is a proton transfer reaction occurring in the presence of a coordinating media—not unlike a proton transfer reaction in solution. For guest exchange to occur, the protonated amino acid and the alkyl amine (the gaseous reagent) must physically come together and exchange a proton causing the amino acid to leave as a neutral. Scheme **3** illustrates a possible mode of interaction involving the β-CD, the amino acid, and the alkyl amine. The amino acid interacts via the carboxylic group, which coordinates with, for example, the upper rim of the cyclodextrin through the C(2)– or C(3)–OCH_3, and the ammonium group, which coordinates with the lower rim through the C(6)–OCH_3. During the exchange, the alkyl amine and the protonated amino acids must come in physical contact to undergo proton transfer. Meanwhile, the R group of the amino acid and the alkyl group of the amine (R') interact with the inner wall of the cyclodextrin cavity to produce enantioselectivity.



Scheme 3

Conclusions

In the gas phase, the three-point interaction model is clearly operational. Although qualitative, this construct offers a simple and straightforward approach to understanding the nature of chiral recognition in oligosaccharide host–guest complexes.

For amino acids interacting with an oligosaccharide host, both the amino and the carboxylic termini can form attractive interactions through hydrogen bonding, whereas the side chain can be either attractive or repulsive, depending on the functional group on the side chain. For the gas phase systems, only one attractive interaction in necessary, provided that it leads to the formation of a relatively stable gas-phase complex. The three-point interaction is either enhanced or mediated by the size of the cavity. In this study and an earlier one [25], molecular modeling calculations predict that inclusion of the amino acid is the preferred mode of interaction even in the gas phase. The protonated amino terminus usually interacts with the narrower rim composed of C(6)–OCH_3. The carboxylic acid may interact with either the narrower or wider rim, depending on the chirality of the amino acid. Even linear oligosaccharides interact with protonated amino acids to produce quasi-inclusion complexes. Unlike cyclodextrins which have relatively fixed dimensions, linear oligosaccharides have significantly expandable cavities that can fit relatively large guests.

Enantioselectivity is enhanced by a cooperative effect between the overall size of the guest and the cavity size of the host. When the guest is small compared to the host cavity, both enantiomers may interact to produce a number of distinct complex structures many of which may have similar reactivities to guest exchange. When the guest is large compared to the host cavity, the number of distinct complex structures decreases significantly as the attractive interactions have to be rectified with the repulsive interaction between the interior of the cavity and the large bulky side chain. For Phe, this produces a single distinct complex structure for both enantiomers leading to the loss of enantioselectivity. When the size of the guest is optimal relative to the cavity, each enantiomer finds a distinct interaction that yields a rate of exchange that is different relative to the other.

Acknowledgments

Funding provided by the National Science Foundation and the National Institutes of Health is gratefully acknowledged.

References

1. Hofmeister, G.; Leary, J. A. *Org. Mass Spectrom.* **1991,** *26*, 811–812.
2. Dang, T. T.; Pedersen, S. F.; Leary, J. A. *J. Am. Soc. Mass Spectrom.* **1994,** *5*, 452–459.
3. Reetz, M. T.; Becker, M. H.; Klein, H. W.; Stöckigt, D. *Angew. Chem. Int. Ed.* **1999,** *38*, 1758–1761.
4. Tao, W. A.; Zhang, D.; Wang, F.; Thomas, P. D.; Cooks, R. G. *Anal. Chem.* **1999,** *71*, 4427–4429.
5. Liang, Y.; Bradshaw, J. S.; Izatt, R. M.; Pope, R. M.; Dearden, D. V. *Int. J. Mass Spectrom.* **1999,** *185/186/187*, 977–988.
6. Sawada, M.; Shizuma, M.; Takai, Y.; Yamada, H.; Kaneda, T.; Hanafusa, T. *J. Am. Chem. Soc.* **1992,** *114*, 4405–4406.
7. Sawada, M.; Takai, Y.; Yamada, H.; Kaneda, T.; Kamada, K.; Mizooku, T.; Hirose, K.; Tobe, Y.; Naemura, K. *Chem. Commun.* **1994**, 2497–2498.
8. Sawada, M. *Mass Spectrom. Rev.* **1997,** *16*, 73–90.
9. Sawada, M.; Takai, Y.; Kaneda, T.; Arakawa, R.; Okamoto, M.; Doe, H.; Matsuo, T.; Naemura, K.; Hirose, K.; Tobe, Y. *Chem. Commun.* **1996**, 1735–1736.
10. Sawada, M.; Takai, Y.; Yamada, H.; Hirayama, S.; Kaneda, T.; Tanaka, T.; Kamada, K.; Mizooku, T.; Takeuchi, S.; Ueno, K.; Hirose, K.; Tobe, Y.; Naemura, K. *J. Amer. Chem. Soc.* **1995,** *117*, 7726–7736.
11. Fales, H. M.; Wright, G. W. *J. Am. Chem. Soc.* **1977,** *99*, 2339–2340.
12. Denisov, E. N.; Shustryakov, V.; Nikolaev, E. N.; Winkler, F. J.; Medina, R. *Int. J. Mass Spectrom. Ion Proc.* **1999,** *183*, 357–368.
13. Honovich, J. P.; Karachevtsev, G. V.; Nikolaev, E. N. *Rapid Commun. Mass Spectrom.* **1992,** *6*, 429–433.
14. Nikolaev, E. N.; Goginashvily, G. T.; Talrose, V. L.; Kostjanovsky, R. G. *Int. J. Mass Spectrom. Ion Processes* **1988,** *86*, 249–252.
15. Nikolaev, E. N.; McMahon, T. B. *43rd ASMS Conference on Mass Spectrometry and Allied Topics*; Atlanta, GA, May 21–26, 1995.
16. Chu, I. H.; Dearden, D. V.; Bradshaw, J. S.; Huszthy, P.; Izatt, R. M. *J. Am. Chem. Soc.* **1993,** *115*, 4318–4320.
17. Dearden, D. V.; Dejsupa, C.; Liang, Y. J.; Bradshaw, J. S.; Izatt, R. M. *J. Am. Chem. Soc.* **1997,** *119*, 353–359.
18. Camara, E.; Green, M. K.; Penn, S. G.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1996,** *118*, 8751–8752.
19. Gong, S.; Camara, E.; He, F.; Green, M. K.; Lebrilla, C. B. *Int. J. Mass Spectrom. Ion Processes* **1999,** *185/186/187*, 401–412.
20. Ramirez, J.; He, F.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1998,** *120*, 7387–7388.
21. Dalgliesh, C. E. *J. Chem. Soc.* **1953**, 3940–3952.
22. Kitae, T.; Nakayama, T.; Kano, K. *J. Chem. Soc., Perkin Trans. 2* **1998**, 207–212.
23. Li, S.; Purdy, W. C. *Chem. Rev.* **1992,** *92*, 1457–1490.
24. Kano, K.; Kitae, T.; Takashima, H. *Inclusion Phenom. Mol. Recognit. Chem.* **1996,** *25*, 243–248.
25. Ramirez, J.; Ahn, S.; Grigorean, G.; Lebrilla, C. B. *J. Am. Chem. Soc.,* **2000,** *122*, 6884–6890.
26. Easson, E. H.; Stedman, E. *Biochemistry* **1933,** *27*, 1257.
27. Ogston, A. G. *Nature* **1948,** *4129*, 963.
28. Sokolov, V. I.; Zefirov, N. S. *Dokl. Akad. Nauk* **1991,** *319*, 1382–1383.
29. Booth, T. D.; Wahnon, D.; Wainer, I. *Chirality* **1997,** *9*, 96–98.
30. Davankov, V. *Chirality* **1997,** *9*, 99–102.
31. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984,** *1984*, 209–217.
32. Hoogwater, D. A.; Peereboom, M. *Tetrahedron* **1990,** *46*, 5325–5332.
33. Gard, E. E.; Green, M. K.; Warren, H.; Camara, E. J. O.; He, F.; Penn, S. G.; Lebrilla, C. B. *Int. J. Mass Spectrom. Ion Processes* **1996,** *158*, 115–127.
34. Carroll, J. A.; Penn, S. G.; Fannin, S. T.; Wu, J.; Cancilla, M. T.; Green, M. K.; Lebrilla, C. B. *Anal. Chem.* **1996,** *68*, 1798–1804.
35. Hunter, E. P. L.; Lias, S. G. *J. Phys. Chem. Ref. Data* **1998,** *27*, 413–656.
36. Still, W. C.; Kilburn, J. D.; Sanderson, P. E. J.; Liu, R.; Wiley, M. R.; Hollinger, F. P.; Hawley, R. C.; Nakamima, M.; Bernardi, A.; Hong, J. I.; Namgoong, S. K. *Isr. J. Chem.* **1992,** *32*, 41–45.
37. Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997,** *97*, 3313–3361.